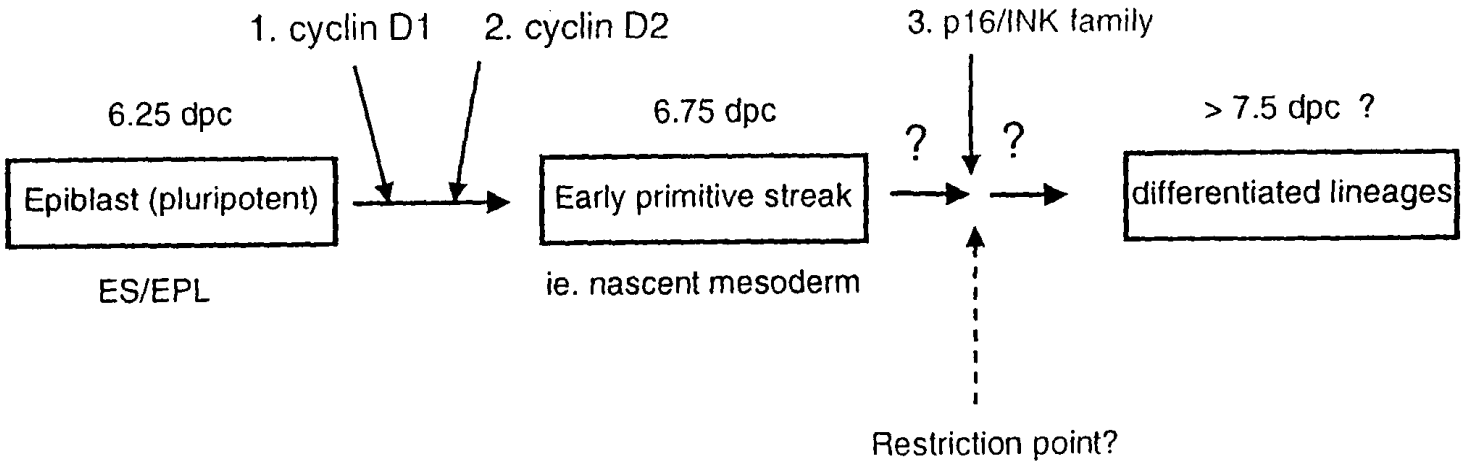
mitogenic signals
active Cdk
E2F
E2F
•activation of target genes
•cell proliferation
early G1
R-point (G1)
G1 → S-phase
Restriction (R-) Point Control
Normal somatic cells
1. Regulated activation of pRb-kinases (Cdks)
2. pRb phosphorylation
3. Derepression of genes required for G1-S progression
Embryonic cells (tumour cells)
1. no regulated pRb kinase (Cdk2-cyclinE)
2. pRb constitutitively hyper-phosphorylated
3. E2F target genes constitutively active

Figure 1

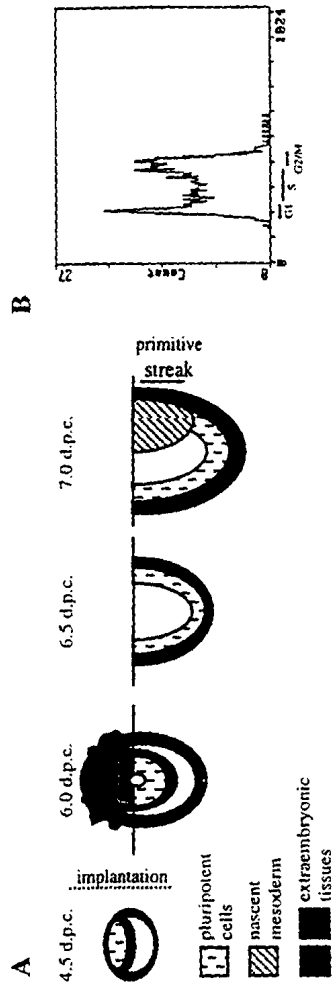
Remodelling of the cell cycle during embryonic development/differentiation



cell cycle length	8 hrs	4-6 hrs rapid proliferative phase	20-24 hrs

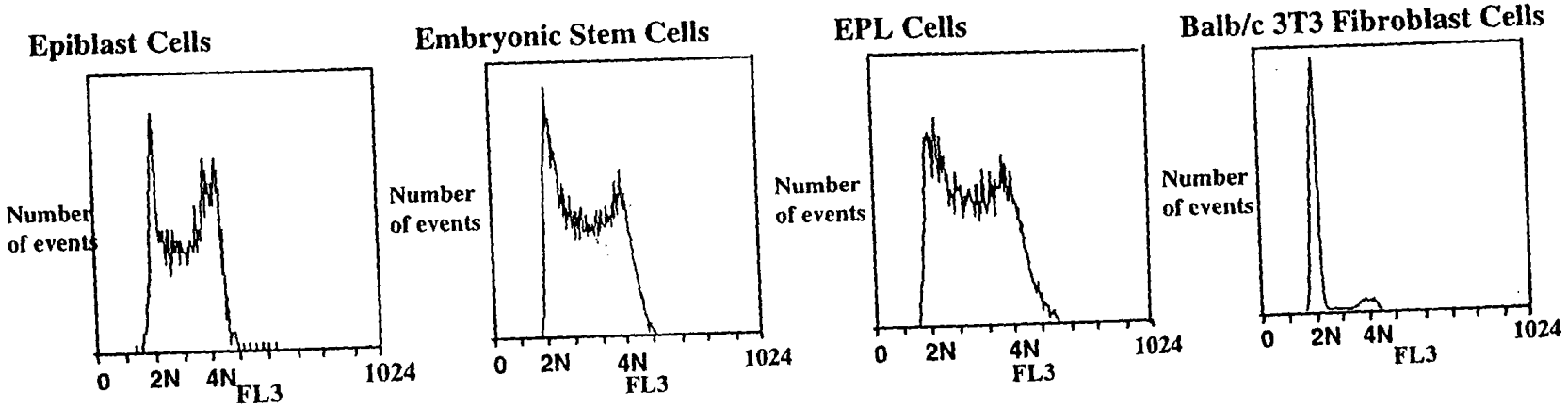
Figure 2

Figure 3



Early mouse embryogenesis. A. Schematic representation of mouse embryogenesis, between 4.5 and 7.0 d.p.c, highlighting the pluripotent cell populations and prior to and during the onset of gastrulation. Gastrulation initiates at the primitive streak and results, initially, in the formation of mesoderm. B. Pluripotent cells from 6.5 d.p.c. embryos were isolated, labeled with propidium iodide and subjected to flow cytometry analysis.

Figure 4


Epiblast Cells
Embryonic Stem Cells
EPL Cells
Balb/c 3T3 Fibroblast Cells
Number of events
0
2N
4N
1024
FL3

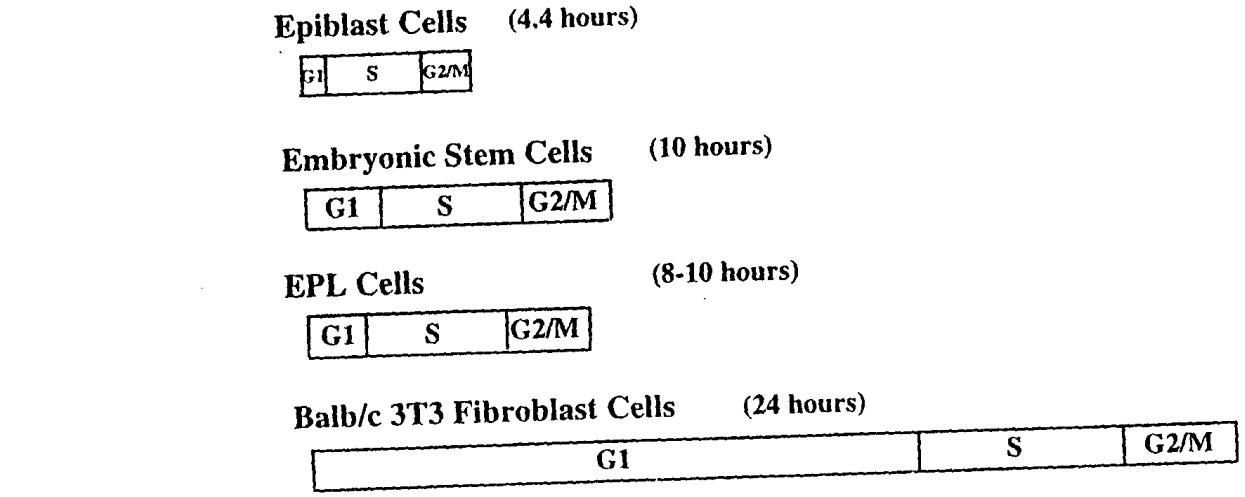

Epiblast Cells (4.4 hours)
G1
S
G2/M
Embryonic Stem Cells (10 hours)
G1
S
G2/M
EPL Cells (8-10 hours)
G1
S
G2/M
Balb/c 3T3 Fibroblast Cells (24 hours)
G1
S
G2/M

Figure 5

Cell cycle remodelling during differentiation of cells in embryoid bodies

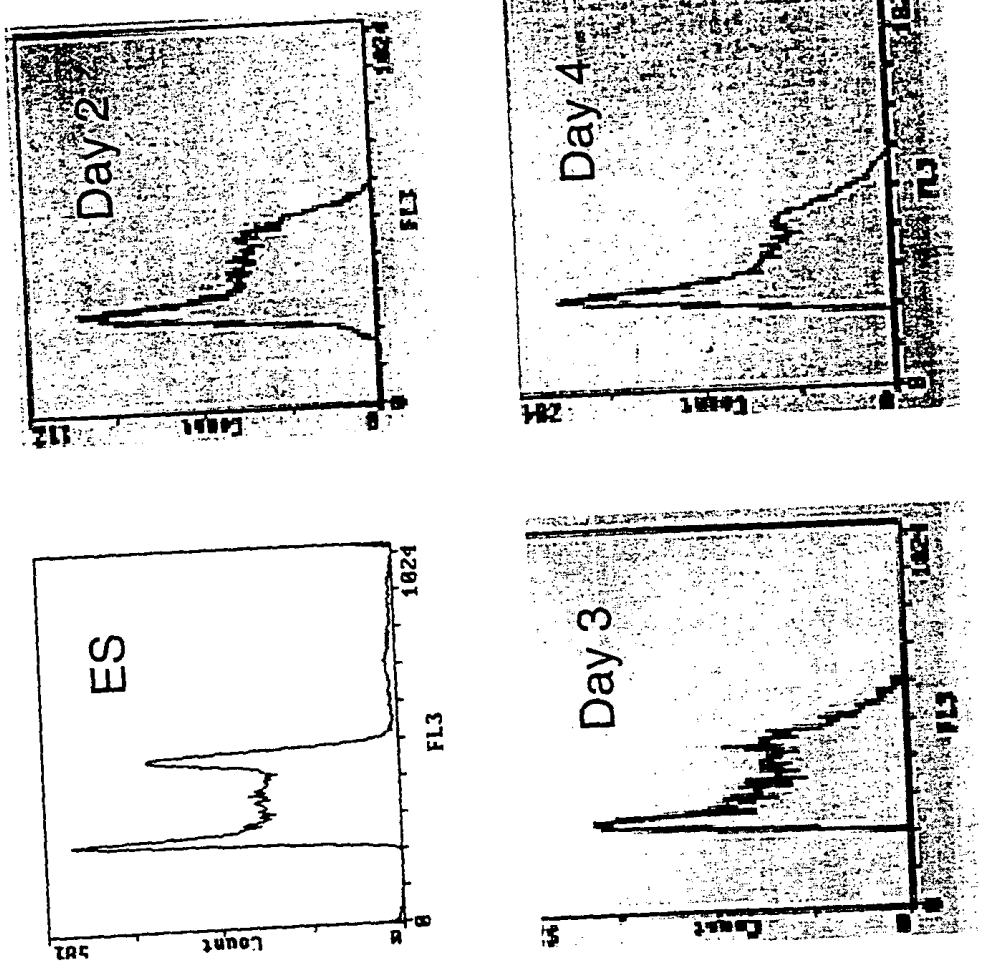
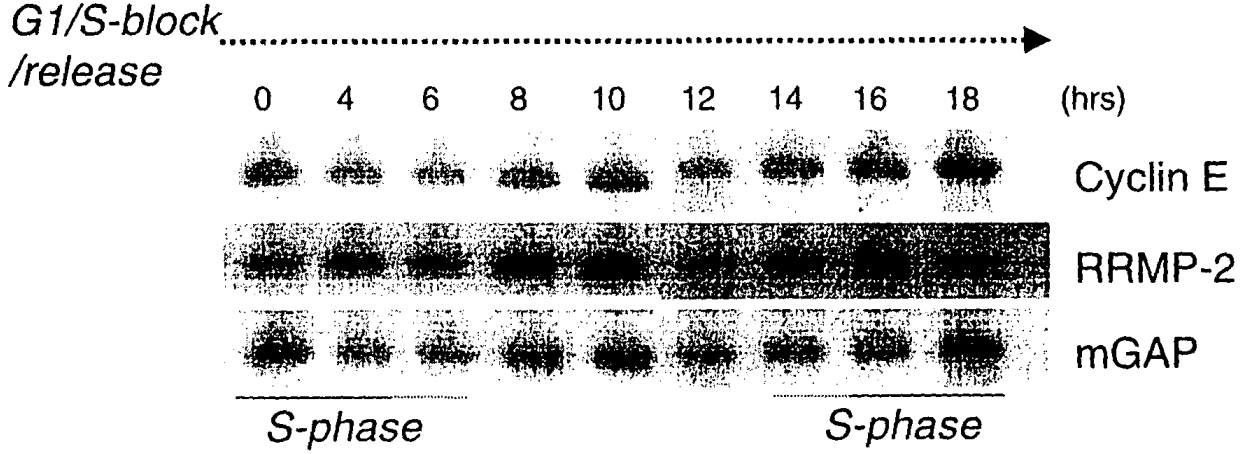


Figure 6

E2F target genes are not cell cycle regulated in ES cells



Cell cycle regulation of E2F transcripts in mouse fibroblasts

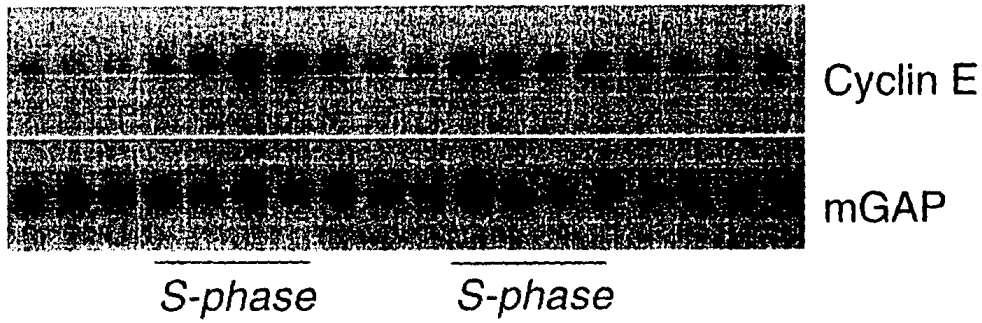


Figure 7

E2F complexes in pluripotent cells are predominantly free of pRb family members

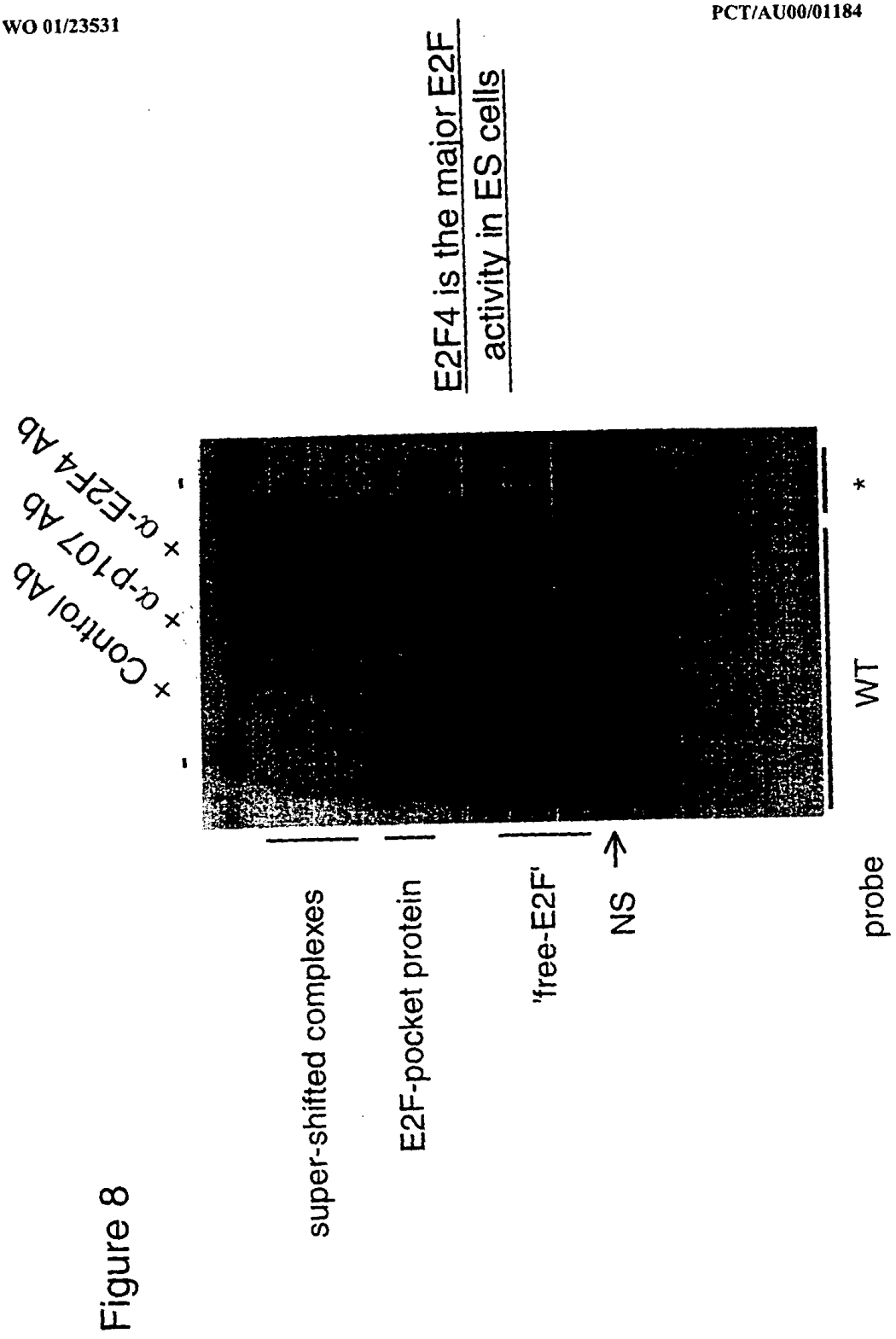

E2F4 is the major E2F activity in ES cells
- + Control Ab
- + α-p107 Ab
- + α-E2F4 Ab
WT
*
probe
NS
'free-E2F'
E2F-pocket protein
super-shifted complexes

Figure 8

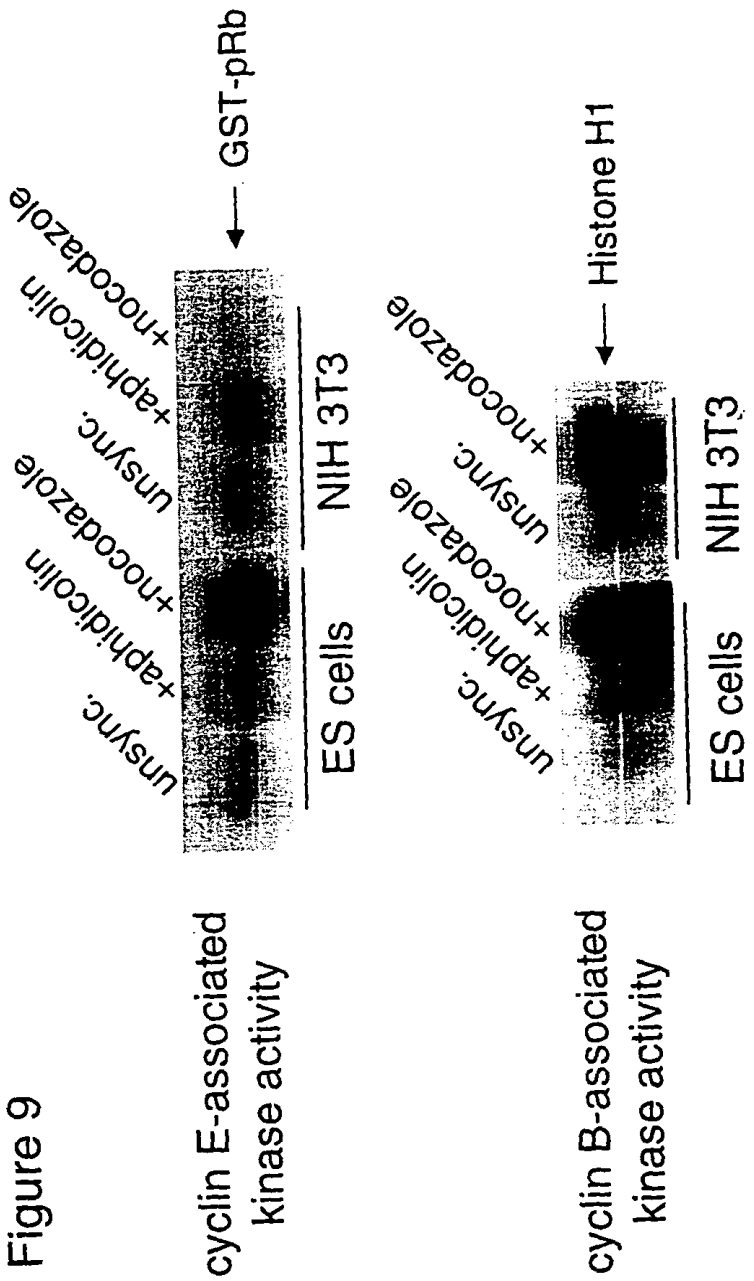

GST-pRb
Histone H1
unsync.
+aphidicolin
+nocodazole
unsync.
+nocodazole
ES cells
NIH 3T3
cyclin E-associated kinase activity
cyclin B-associated kinase activity

Figure 9

Figure 10

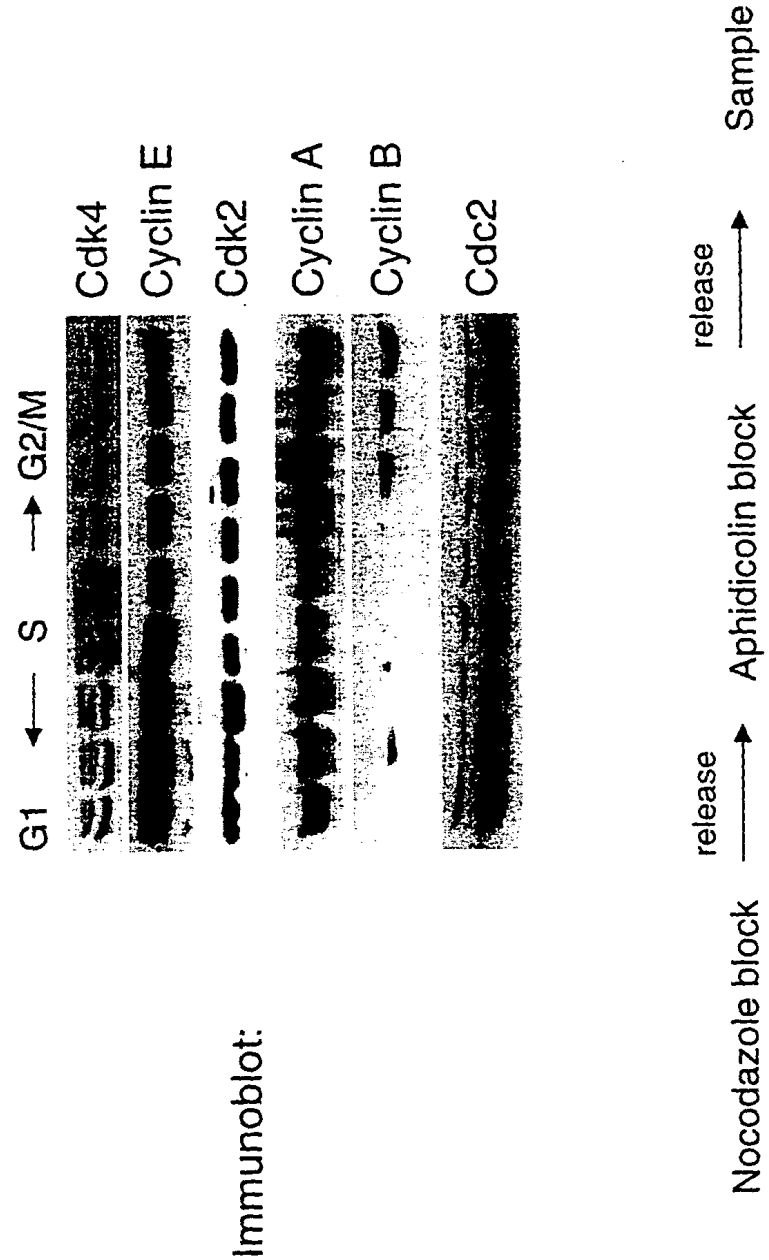
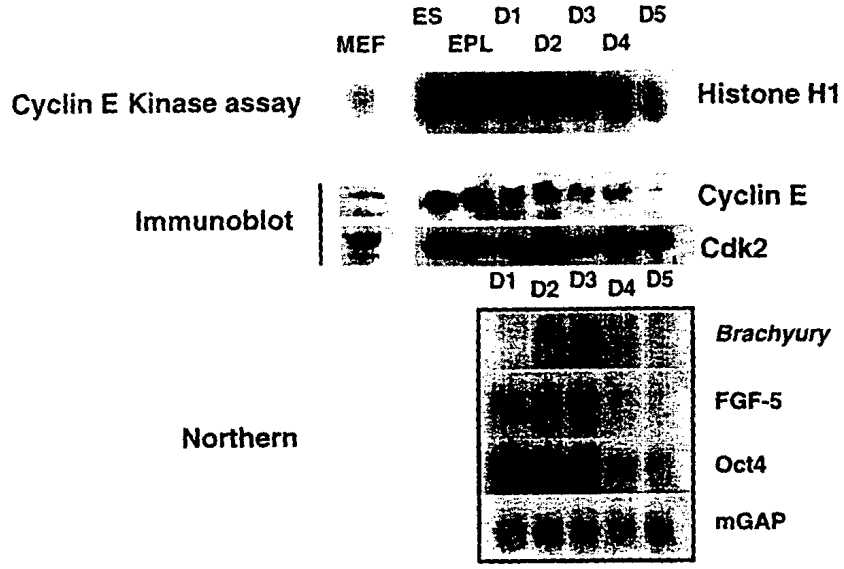

Immunoblot:
G1
S
G2/M
Cdk4
Cyclin E
Cdk2
Cyclin A
Cyclin B
Cdc2
Nocodazole block
release
Aphidicolin block
release
Sample

Figure 11



ES ⟶ EPL ⟶ EPL embryoid bodies ⟶ mesoderm

Cyclin E and cyclin A associated kinase activities collapse during differentiation

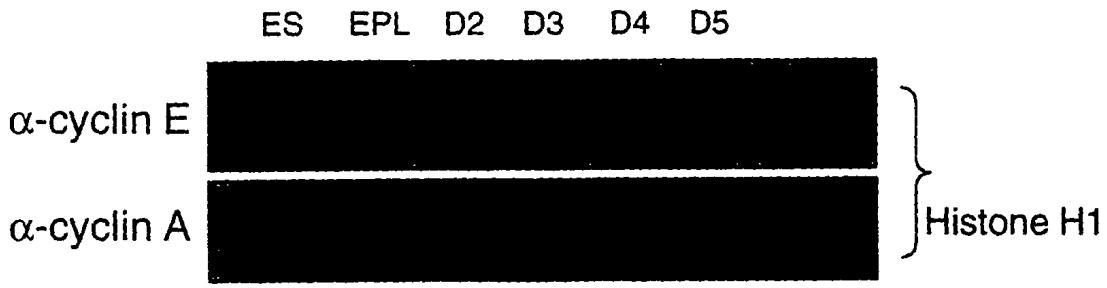


Figure 12

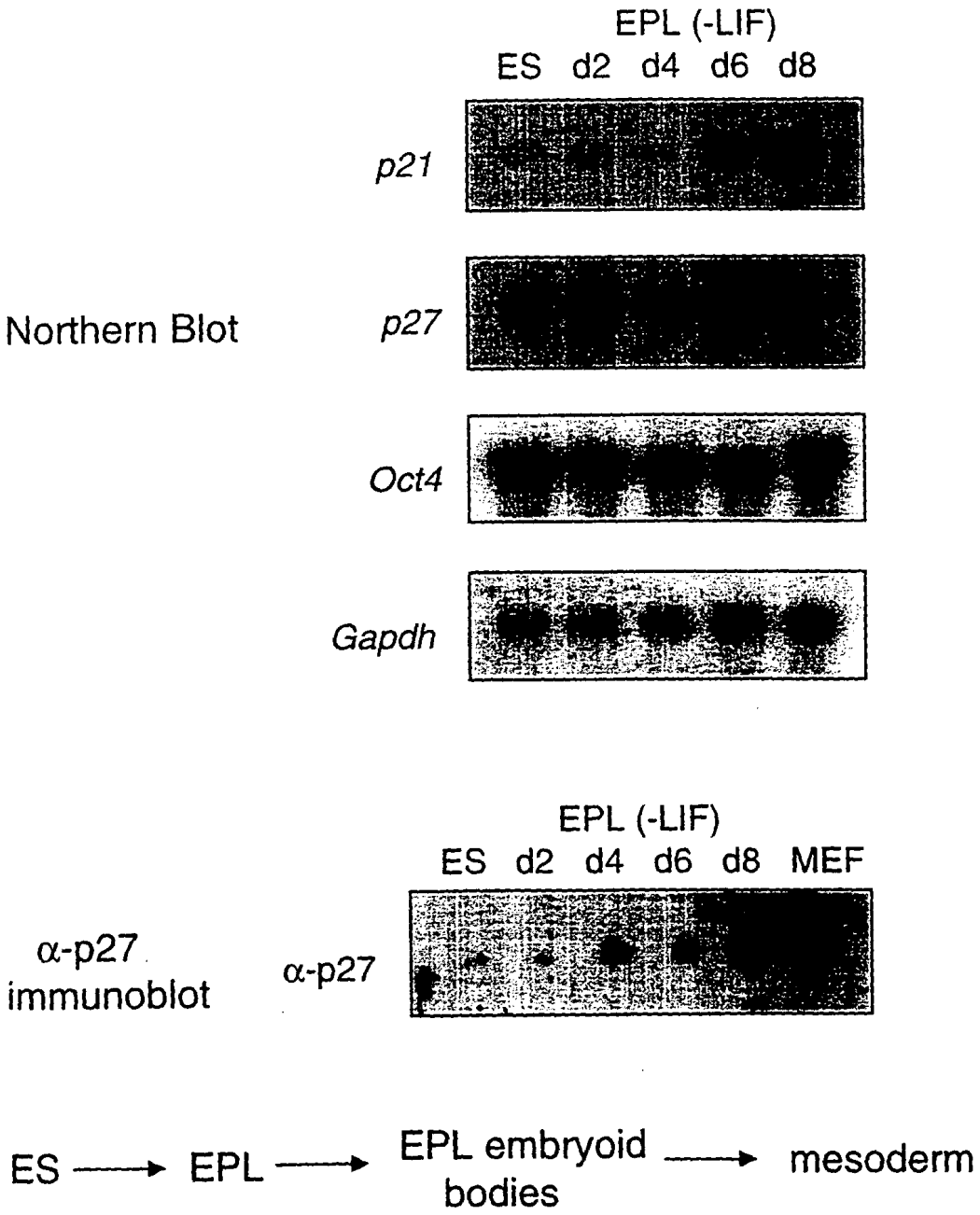

EPL (-LIF)
ES d2 d4 d6 d8
p21
p27
Northern Blot
Oct4
Gapdh
EPL (-LIF)
ES d2 d4 d6 d8 MEF
α-p27
immunoblot
α-p27
ES → EPL → EPL embryoid bodies → mesoderm

Figure 13

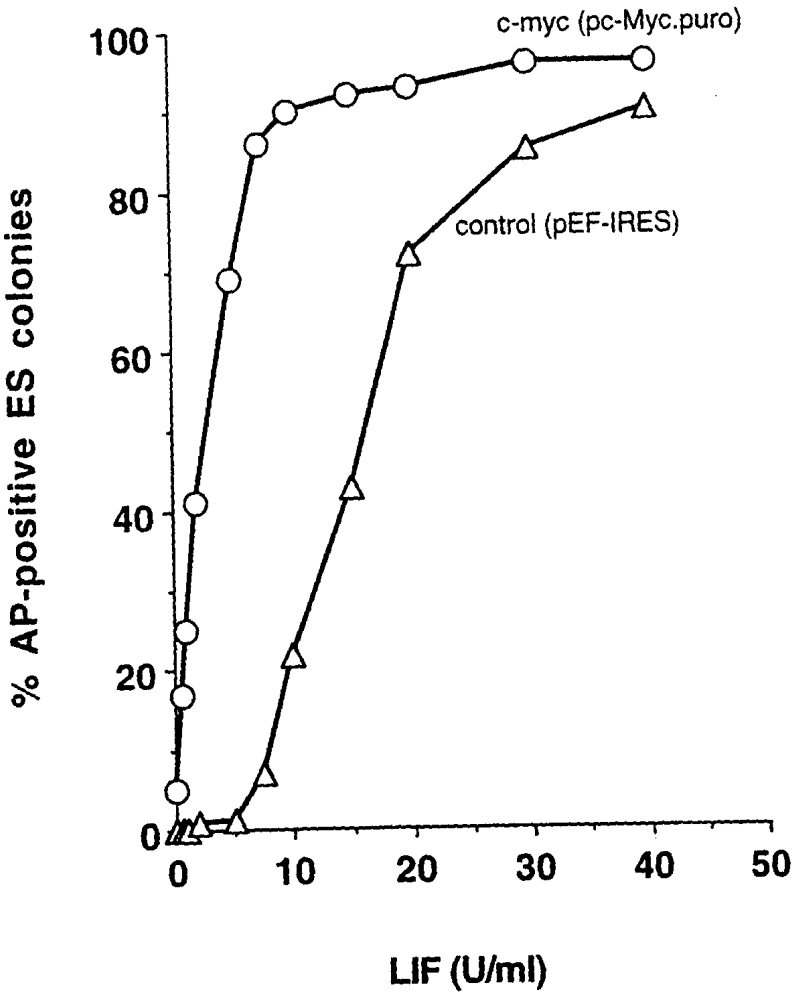
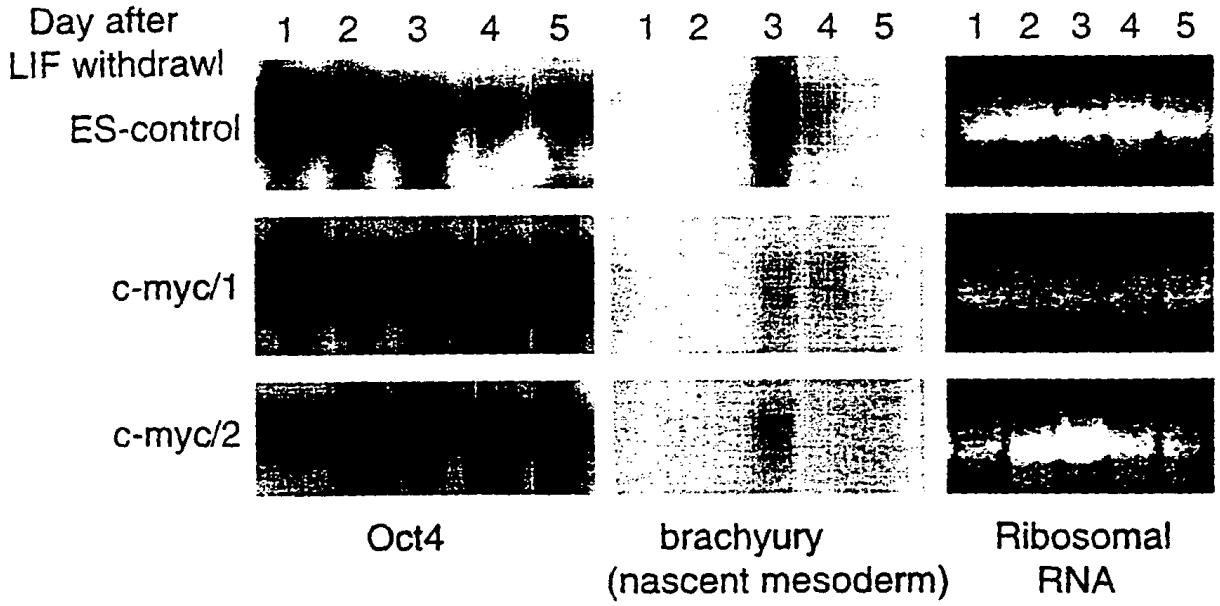
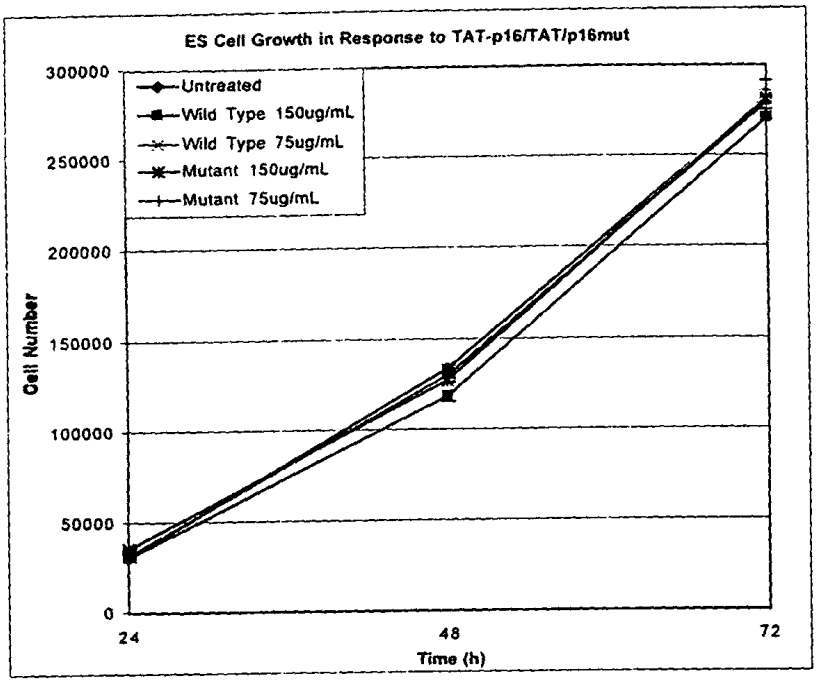

c-myc (pc-Myc.puro)
control (pEF-IRES)
% AP-positive ES colonies
LIF (U/ml)
0
20
40
60
80
100
0
10
20
30
40
50

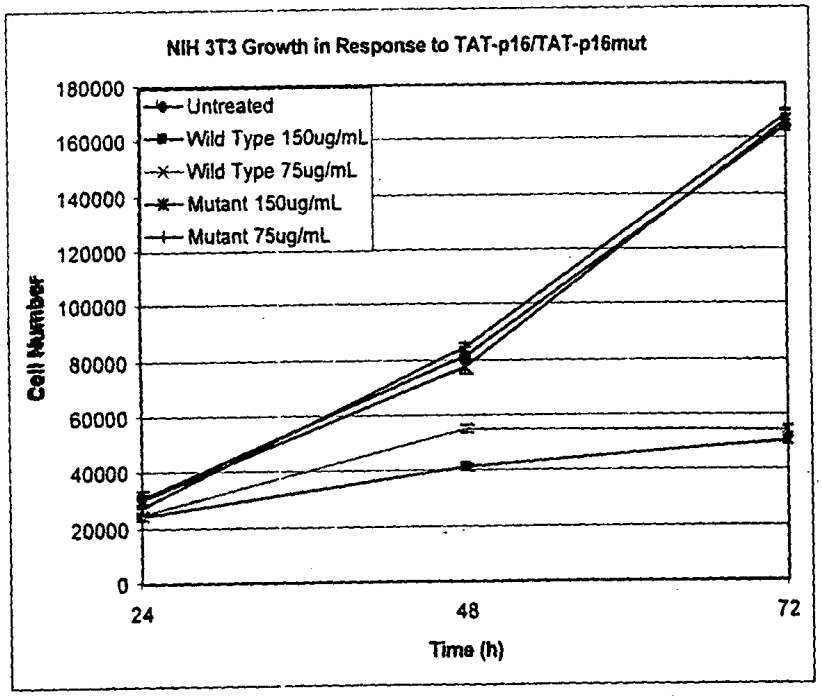
Figure 14



ES ⟶ EPL ⟶ EPL embryoid bodies ⟶ mesoderm

Figure 15


ES Cell Growth in Response to TAT-p16/TAT/p16mut
Untreated
Wild Type 150ug/mL
Wild Type 75ug/mL
Mutant 150ug/mL
Mutant 75ug/mL
Cell Number
300000
250000
200000
150000
100000
50000
0
24
48
72
Time (h)


NIH 3T3 Growth in Response to TAT-p16/TAT-p16mut
Untreated
Wild Type 150ug/mL
Wild Type 75ug/mL
Mutant 150ug/mL
Mutant 75ug/mL
Cell Number
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
24
48
72
Time (h)